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GENE-ENZYME VARIATION IN THREE SYMPATRIC SPECIES OF *LITTORINA*. II. THE ROSCOFF POPULATION, WITH A NOTE ON THE ORIGIN OF NORTH AMERICAN *L. LITTOREA*

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The three sympatric species of *Littorina* inhabiting the north Atlantic coasts offer an interesting system for evaluating the effect of migration on the genetic structure of species' populations. *Littorina littorea*, the large common periwinkle, inhabits the mid-intertidal region, and during its reproductive cycle it deposits fertilized eggs directly to sea where development proceeds pelagically for several weeks (Purchon, 1968). Larvae, extensively dispersed by longshore currents, then find their way ashore and metamorphose into juvenile adults. *Littorina saxatilis*, in contrast, resides in the upper-intertidal and spray zone (Haseman, 1911), and is ovoviviparous. Fertilized eggs are retained within the female and the emerging form is the juvenile adult (Purchon, 1968). In terms of larval dispersal and gene flow, then, *L. saxatilis* is quite restricted. The third species, *Littorina obtusata*, inhabits the lower-intertidal region and is a prominent grazer on *Fucus* and *Ascophyllum* (Haseman, 1911). Female specimens of *L. obtusata* cement large egg masses onto the anchored algae, and development proceeds there to the emerging juvenile adult stage. Dispersal in this species is limited to rafting on dislodged algae.

The outcome of a genetic analysis of 15 sympatric populations of the three species collected along a 700 mile transect from Charlottetown, Prince Edward Island to the southern tip of Cape Cod, Massachusetts was reported several years ago (Berger, 1973). The major conclusion of that study, in which electrophoretic variation was followed at three nonspecific esterase loci, was that, in the two species lacking the pelagic larval stage, genetic differences could be observed at some loci between groups of populations separated by major geographic barriers (Cape Cod, and the unfavorable surface currents around Halifax). Populations within a region (for example, south of the Cape Cod Canal) were genetically similar, in general. The differences between regions, or genetic neighborhoods, came in two forms. There was either a rather sharp quantitative change in allele frequency across some boundary, or else a qualitative change occurred, where a

totally new allele appeared in a region. In *Littorina littorea* the three loci were largely monomorphic in most populations; however, those rare alleles that were detected appeared at comparable frequencies throughout the range.

The results of this first survey conformed with and complemented the patterns of variation observed in other studies. For *Nassarius obsoletus* (Gooch, Smith, and Krupp, 1972), another gastropod mollusc with a lengthy pelagic larval period, and thus extensive dispersal capabilities, allele frequencies at two polymorphic loci were found to be remarkably similar among populations collected along a 1000 mile transect from Cape Cod, Massachusetts, to Beaufort, North Carolina. In contrast, allele frequencies at two polymorphic loci in *L. saxatilis* showed considerable inter-population heterogeneity over even short distances (Snyder and Gooch, 1973).

In this report the results of several additional population genetic studies on the *Littorina* species are presented. The major work concerned an analysis and comparison of electrophoretic variation at ten to thirteen loci in natural populations of the three species collected from Cape Cod, Massachusetts, and Roscoff in Brittany, France. The purpose of this study was to determine the extent to which very long distances, across open seas, magnify the genetic differences between populations of species capable of either extensive or limited gene flow. A second study examined the temporal stability of allele frequency in several of the North American populations described in the earlier work, to determine whether the genetic differences found between populations north and south of the Cape Cod Canal were constant features of the species population structure.

Materials and Methods

Collection and preparation of specimens

Individual snails were collected at low tide and stored in perforated plastic bags containing moist seaweed until they could be processed for electrophoresis. If kept cool and aerated, the animals could remain completely viable for at least a week. In preparing the animals for electrophoresis, the shell was cracked and the soft parts (hepatopancreas and gonads mainly) were removed and homogenized on ice in two volumes of cold 10% sucrose. The homogenates were centrifuged for 10 minutes at $6000 \times g$, and aliquots of the supernatant were either applied directly to the gels for electrophoresis, or stored frozen at $-18°$ C before analysis.

Electrophoresis

Procedures for 5% acrylamide gel electrophoresis, and for the localization of esterase (Est) activity, or general protein (Pt) followed the method of Hubby and Lewontin (1966). In studies utilizing 12.5% starch gel electrophoresis (Selander, Smith, Yang, Johnson, and Gentry, 1971), either of two buffer systems were used. The tris-citrate system of Nichols and Ruddle (1973) served for the analysis of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), hexokinase (Hex), tetrazolium oxidase (TO), 6-phosphogluconate dehydrogenase (Pgd), glucose-6-phosphate dehydrogenase (Zw), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), amino peptidases (AP), and leucine aminopeptidase (LAP). Electrophoresis was carried out for 16 hours at 85 V. For esterase (Est), the discontinuous Poulik system was used (Selander *et al.*, 1971). The staining pro-

TABLE I

Gene enzyme variation in natural populations of Littorina saxatilis *from Roscoff and Cape Cod. Allele designations are in order of increasing relative mobility, where A represents the slowest migrating allozyme (most cathodal). Sample size (N) is noted for each locus. At six loci (TO, LDH, MDH, Hex, Pdg, Zw) the two populations were monomorphic for the same allele.*

Locus	Site	N	Allele frequency							
			A	B	C	D	E	F	G	H
Est-C	Rosc.	69	0.76	0.24						
	C. Cod	45	0.68	0.32						
LAP-1	Rosc.	72	0.96	0.04						
	C. Cod	36	1.0	0						
LAP-2	Rosc.	54	1.0	0						
	C. Cod	36	0.96	0.04						
PGI	Rosc.	71	0.01	0.24	0.62	0.11	0.01	0.01		
	C. Cod	47	0	0.14	0.03	0.83	0	0		
Est-A	Rosc.	67	0	0	0.01	0.04	0.07	0.49	0.26	0.14
	C. Cod	46	0.12	0.33	0	0	0.03	0	0.42	0.09

cedures for these various enzymes were from Selander *et al.* (1971) and Shaw and Prasad (1970).

RESULTS

The Roscoff and Cape Cod populations

Between 11 and 13 gene-enzyme systems were examined by acrylamide and starch gel electrophoresis in the three species, from single collections made in Brittany, France, and Cape Cod, Massachusetts. The results of this analysis are presented in Tables I through III.

In *L. saxatilis* (Table I) 11 enzyme loci were examined. At six of these (TO, LDH, MDH, Hex, Pgd, Zw) the two populations were found to be monomorphic, and in each case allozymes in the two populations were indistinguishable by electrophoresis. Sample sizes ranged from 40–47. At three loci (Est-C, LAP-1, LAP-2) either one or both populations were polymorphic and allele frequencies in both were quite similar. At the PGI locus the Roscoff population was found to be segregating six different alleles; however, three were at very low frequency. In the Cape Cod population only the three most common alleles were found, but at frequencies quite different from the Roscoff collection. Only at EST-A did there appear to be important qualitative genetic differences between the two populations: the Roscoff population was found to maintain six distinct alleles, and the Cape Cod population maintained five. However, only three of the total of eight alleles were shared. The average heterozygosity value calculated over the 11 loci in the Roscoff population was 0.15, and in the Cape Cod population it was 0.12.

In *L. obtusata* a total of 13 loci were examined by a combination of starch and acrylamide gel electrophoresis. At seven loci (LDH, MDH, Hex, AP-1, AP-2,

Table II

Gene-enzyme variation in natural populations of Littorina obtusata *from Roscoff and Cape Cod. Allele designations are in order of increasing relative mobility, where A is slowest migrating allozyme at that locus. At seven loci (LDH, MDH, Hex, AP-1, AP-2, Pt-1, LAP-2) both populations were fixed for the same allele.*

Locus	Site	N	Allele frequency						
			A	B	C	D	E	F	G
PGI	Rosc.	72	0	0.01	0.27	0	0.64	0.01	0.07
	C. Cod	9	0.35	0	0.15	0.35	0.15	0	0
Est-3	Rosc.	65	0.16	0.37	0.43	0.01	0.02		
	C. Cod	37	0	0.40	0.60	0	0		
Est-1	Rosc.	79	0.79	0.21					
	C. Cod	45	0.58	0.42					
Est-4	Rosc.	18	0.78	0.08	0.11	0.03			
	C. Cod	26	0.94	0.06	0	0			
Pt-2	Rosc.	8	0.12	0.88					
	C. Cod	23	1.00	0					
LAP-1	Rosc.	54	0.02	0.96	0.02				
	C. Cod	24	0	1.00	0				

Pt-1, LAP2; N = 16–64) both populations appeared monomorphic for the same allele. At the remaining loci (Table II), either one or both populations were polymorphic. For LAP-1 Est-4, Est-3, and Est-1 both populations maintained the same alleles at high frequency, although in three cases the Roscoff population had low frequency alleles present which were absent at Cape Cod. At the Pt-2 locus, the two populations shared alleles in common, but at Roscoff a unique allele was at high frequency. Only at PGI did there appear to be significant genetic differences between the two populations, although, even here, two of the seven total alleles are commonly shared. Values of heterozygosity in the Roscoff and Cape Cod populations were 0.17 and 0.13, respectively.

Twelve gene-enzyme systems were examined in *L. littorea* (Table III). Of these, three (AP-2, LAP-1, LDH) were monomorphic in both populations, for the same allele (N = 12–72). At two loci, Est-A and LAP-2, the two populations shared an allele in common, but this allele was always rare in Roscoff, and fixed in Cape Cod. At seven loci, the two populations were completely different genetically. Calculated values of average heterozygosity for the Roscoff and Cape Cod populations were 0.15 and 0.03, respectively.

Temporal variation

When two geographically separate populations are found to differ genetically, it becomes important to examine their temporal stability if one wishes to explain those differences by an argument involving gene flow. Table IV summaries the results of allozyme analysis on several populations taken over a number of years. The

three loci studied here correspond to those described in an earlier report (Berger, 1973).

For the most part, allele frequencies in *Littorina* populations have stayed rather constant from year to year. There are several obvious exceptions, especially *L. obtusata* at Rockland, where significant differences were apparent. At the Maine sample, however, the collections made over the two years were done at different beach sites. This could account for the large variation. Nevertheless, the general qualitative and quantitative differences that were reported for the 1972 collection (the absence of *L. obtusata* Est-1$^{8'}$ allele, and *L. saxitalis* Est-3$^{8'}$ allele south of the Cape Cod Canal) still appear to be present, and apparently represent long-term features of these species' population structures.

Microgeographic variation in L. obtusata

In the Cape Cod region *L. obtusata* can range in shell color from a light yellow (almost white) to ebony. Shell colors of several intermediate hues (orange, brown) can be discerned, and the presence of distinctly striped individuals suggests

TABLE III

Gene-enzyme variation in natural populations of Littorina littorea *from Roscoff and Cape Cod. Allele designations are in order of increasing relative mobility, where A is the slowest migrating (most cathodal) allozyme at that locus. Sample size (N) is noted for each locus. At three loci (AP-2, LAP-1, LDH) both populations were fixed for the same allele.*

Locus	Site	N	Allele frequency						
			A	B	C	D	E	F	G
PGI	Rosc.	71	0.02	0.03	0.77	0.18	0	0	0
	C. Cod	62	0	0	0	0	0.01	0.02	0.97
PGM1	Rosc.	65	0	0	0.06	0.35	0.59		
	C. Cod	44	0.08	0.92	0	0	0		
PGM2	Rosc.	67	0	0.08	0.91	0.01			
	C. Cod	72	1.00	0	0	0			
AP-1	Rosc.	12	1.00	0	0				
	C. Cod	12	0	0.54	0.46				
Est-C	Rosc.	71	0.62	0.38	0				
	C. Cod	63	0	0	1.00				
AP-3	Rosc.	32	1.00	0					
	C. Cod	16	0	1.00					
MDH	Rosc.	32	1.00	0					
	C. Cod	16	0	1.00					
Est-A	Rosc.	70	0.04	0.02	0.54	0.40			
	C. Cod	63	1.00	0	0	0			
LAP-2	Rosc.	72	0.98	0.02					
	C. Cod	16	0	1.00					

TABLE IV

Four year survey of Esterase polymorphisms in three littorinid species.

Locale	Sample Size	Year	*L. obtusata*								*L. saxatilis*					*L. littorea*			
			Est-1			Est-3			Est-4		Est-3*			Est-4		Est-3*		Est-4	
			F	S	S′	F	S	O	F	S	F	S	S′	F	S	F	S	F	S
Woods Hole	22–33	1972	0.44	0.56		0.35	0.65		0.48	0.52	0.47	0.53		0.05	0.95	0.92	0.08		1.0
	24–47	1973	0.43	0.57		0.27	0.73		0	1.0	0.47	0.53			1.0	1.0			1.0
	20–41	1975	0.44	0.56		0.44	0.56		0	1.0	0.53	0.47		0.01	0.99	0.99	0.01		1.0
	16–30	1976	0.41	0.59		0.38	0.62		0	1.0	0.42	0.58		0	1.0	1.0			1.0
Manomet	23–24	1972	0.22	0.78		0.40	0.60		0.08	0.92	0.19	0.54	0.27	0.02	0.98	1.0			1.0
	16–47	1973	0.13	0.83	0.03	0.56	0.44		0.03	0.97	0.40	0.53	0.07		1.0	1.0			1.0
	37–41	1976	0.06	0.86	0.08	0.36	0.64		0	1.0	0.18	0.60	0.21		1.0	1.0			1.0
Rockland / Bar Harbor	13–24	1972	0.37	0.63		0.31	0.69		0	1.0	0.10	0.57	0.33	0.14	0.86	1.0			1.0
	14–24	1973	0.06	0.94		0.54	0.37	0.09	0	1.0	0.18	0.59	0.23	0.12	0.88	1.0			1.0
Prince Edward Island	5–28	1972	0	0.41	0.57	0.15	0.85		0	1.0	0.51	0.34	0.15	0.01	0.99	0.92	0.08		1.0

* In 1973 high resolution electrophoresis detected heterogeneity among one of the allelic classes at each of two loci. However, this variation could not be scored unambiguously and the two variants found within the *Est-3*^{F} of *L. littorea* and *Est-3*^{S} of *L. saxatilis* are pooled.

TABLE V

Microgeographic variation of shell color and enzyme phenotype in Littorina obtusata, *based on data collected in August, 1972. Collections were made from Quisset Harbor and Gansett Point, near Woods Hole.*

Site	N	Est-1			Est-3		Shell color		
		F	S	O	F	S	N	Yellow (%)	Brown (%)
Gansett	24	0.54	0.44	0.02	0.48	0.52	35	0.06	0.89
Quisset-A	44	0.48	0.51	0.01	0.36	0.64	275	0.20	0.78
Quisset-B	20	0.55	0.43	0.02	0.39	0.61	144	0.38	0.55
Quisset-C	48	0.53	0.46	0.01	0.48	0.52	200	0.23	0.74
Quisset-D	48	0.53	0.46	0.01	0.42	0.58	125	0.16	0.83
Quisset-E	45	0.52	0.50	0	0.41	0.59	86	0.27	0.73

that the basis for shell color polymorphism is genetic. Superimposed on the color variation is an obvious polymorphism for band thickness in the striped individuals. In Manomet, for example, one finds that striped shells consist of a large number of very fine pigment bands, while at Nobska, striped shells contain only four or five broad bands of alternating color.

One striking observation made repeatedly is that the frequencies of shell color variants in the population can change dramatically over even short distances. Since it has already been shown for *L. saxatilis* that shell color probably serves as protection against predators (Heller, 1975) and, therefore, can be under strong selection, it was of interest to look for any possible nonrandom association between shell color and allozyme frequency. This association, if it existed, need not be direct; that is, the enzyme under study would not have to participate in pigment formation. Rather, such nonrandom association could arise by a form of genetic drift established when strong selection for a particular shell color genotype is, by chance, associated with one or more allozyme alleles.

In one locality, Quisset Harbor, the microgeographic cline in shell color frequency was particularly striking. As one moved from the exposed beach at Gansett to the secluded harbor at Quisset, about one-half mile, the frequency of yellow morphs increased from 6% to 38% (Table V). Snails were collected at random from six sites along the transect, and esterase allele frequencies at two loci were examined. As seen in Table V, despite the variation present with respect to shell color, allozyme frequencies were quite similar at all the sites. This observation was confirmed by carefully analyzing shell color and enzyme genotype in a large number of snails collected from Quisset Harbor, Manomet Point, and Nobska (data not shown).

DISCUSSION

The principle aim of the work on *Littorina* has been to evaluate the effect of larval dispersal on the genetic structure of gastropod species. The phenotypic markers which have been followed are allozymes, because they are easy to analyze in large numbers of individuals and can be readily and unambiguously converted into genotype and allele frequencies. Although formal genetic analyses have not

been carried out on these snails, it has become customary to equate electrophoretic banding patterns with genotypes when the population contains a distribution of one-banded (homozygotes), and two- or three-banded (heterozygotes) individuals in Hardy-Weinberg proportions.

Assuming that adult specimens of *Littorina* are sessile, the obvious expectation is that any two populations of *L. littorea*, because of its pelagic larvae, should be more alike genetically than sympatric populations of *L. obtusata* or *L. saxatilis*. Furthermore, increasing distance should magnify the between-population differences in all three species. While this general pattern was observed in early studies examining gastropod populations along the North American coast (Gooch *et al.*, 1972; Berger, 1973; Snyder and Gooch, 1973), the comparison of North American and French *Littorina* shows quite the reverse. The basis for this unpredicted result, I believe, involves the probable origin of North American *L. littorea*. I will deal with this issue first, and return briefly to the general question of gene flow in turn.

The unintentional transport of animals beyond their normal geographic range has always fascinated zoogeographers. One impressive and recent case involves the introduction of the common periwinkle, *Littorina littorea,* to the rocky intertidal coast of New England. *L. littorea* was first recorded in North America at Pictou, Nova Scotia, in 1840 (Ganong, 1886). Later accounts document the subsequent and rapid southern expansion of this species' habitat range (Morse, 1880; Wells, 1965). Today *L. littorea* occurs from Labrador to Maryland and is one of the most abundant members of the intertidal mollusc community (Bequaert, 1943).

The precise origin of North American *L. littorea* has been debated for over 90 years. Ganong postulated that *L. littorea* was introduced by European colonists at the beginning of the century, and that its rapid southern expansion from Nova Scotia reflects its active dispersal capabilities (see Berger, 1973) and its success in comparable temperate habitats along the European coast. His argument is that careful malacological records of New England and the Gulf of St. Lawrence fail, entirely, to include this species, although the related species *L. palliata* (*obtusata*) and *L. rudis* (*saxitalis*) are routinely found. Moreover, no trace of *L. littorea* had been reported from any North American indian shell heap, or from any Post-Pliocene deposits of Greenland, Labrador, Canada, or New England, while shells of other *littorinid species* were common.

Clarke and Erskine (1961) have reported the discovery of several *L. littorea* shells from Micmac shell heaps near Halifax, Nova Scotia, which were determined by carbon dating to be about 700 years old. In order to explain the recent pattern of habitat expansion, Clarke and Erskine proposed that *L. littorea* was native to the Halifax area before the advent of European culture. Its failure to spread prior to the middle of the 19th century was attributed to unfavorable oceanographic factors, specifically, that the major surface currents around Halifax move eastward, out to sea, establishing a formidable barrier to the southern movement of pelagic eggs or larvae. With the advent of commercial shipping between the Maritime Provinces and southern Nova Scotia or New England, passive transport of *L. littorea* occurred, permitting colonization to begin. Further expansion was mediated by the favorable long-shore currents in the region.

A partial solution to this controversy appears to emerge from an examination of the allozyme data in Tables I–III. For *L. obtusata*, at 8 of 13 loci, both populations maintained the same allele or alleles. At the remaining five loci both populations shared alleles in common, but showed site-specific alleles as well. At no locus were the two populations completely different genetically. A similar picture emerges from an analysis of the *L. saxatilis* data. In contrast, at 7 of 12 loci examined, the two populations of *L. littorea* were completely different genetically. At the remaining 5 loci, alleles common to both populations were found. The data are in support of Clarke and Erskine's (1961) hypothesis that, indeed, *L. littorea* is probably an aboriginal species to North America, and that a very recent European origin is unlikely.

One final point of interest emerges from a calculation of average heterozygosity (Lewontin and Hubby, 1966) in these populations. This value is simply calculated by dividing the sum of all expected heterozygote frequencies by the total number of loci examined. For *L. obtusata* (*L. saxatilis*), the values for Roscoff and Cape Cod are comparable—0.17 (0.15) and 0.13 (0.13), respectively. For *L. littorea* the values for Roscoff and Cape Cod are quite different—0.15 and 0.03, respectively. This low value for Cape Cod *L. littorea* indicates a founder effect; reduced variability produced by a small initial sample of colonists. Thus, one may conclude that the rather profound genetic differences and levels of heterozygosity found between *L. littorea* from Europe and North America reflect an ancient population divergence and severe bottleneck in the North American population size. The lack of allele overlap between the two populations is, in fact, comparable to that between different species (Lewontin, 1974). It is unlikely that an entirely acceptable explanation can ever be made for the recent release of this species from Nova Scotia, since shipping along the coast of North America must have occurred prior to the nineteenth century.

Since *L. littorea* is characterized by the most extensive dispersal capabilities among the three species studied, and yet shows pronounced genetic differences between coasts, it is unlikely that significant gene flow occurs across the Atlantic for any species of *Littorina*. Thus, investigations into the effect of dispersal must be limited to studies along the Atlantic coasts. On that count, it is of importance to note that populations of all three species are characterized by temporal constancy of gene frequency. Practically, this allows one to compare geographically separate populations at any instant and evaluate the effect of space unconfounded by genetic changes which may occur over time. Certainly this interesting system of sympatric species may allow for an evaluation of gene flow on population genetic structure, internally controlled for phylogenetic similarity and gene homology.

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Summary

Gene-enzyme variation was examined at ten to fifteen loci in three sympatric species of *Littorina* collected from Cape Cod, Massachusetts and Roscoff, Brittany. The North American and French populations of *L. saxatilis* and *L. obtusata* were, in general, quite similar in allele content and frequency. In contrast, the North American and French populations of *L. littorea* were genetically differentiated at a majority of their loci. This pattern of heterogeneity in the three species, along with calculated values of average heterozygosity, suggests that North American specimens of *L. littorea* are not recent colonists from Europe and have passed through a severe population bottleneck on the North American continent. Survey studies carried out over a four year period revealed a general pattern of temporal constancy in allele frequency in all three species.

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